Axcelead Solutions for Innovative Targeted Protein Degrader

Axcelead Drug Discovery Partners, Inc.

May 2024

Axcelead's Platform of Targeted Protein Degradation | DegLead

Bifunctional degrader

- ***High-throughput parallel synthesis*** *using our* ***degrader chemical toolbox***
- ***Original binders*** *for* ***over hundreds*** *of drug targets*
- *Rapid oral drug discovery by* ***ADME solution***

Molecular glue degrader (MGD)

- ***MGD-focused*** *compound library*
- *Identification of* ***neo-substrate***
- *Comprehensive* ***selectivity*** *evaluation*

Degrader evaluation platform

Screening

- *HiBiT®, Cell imaging assay etc.*
- *NanoBRET®, TR-FRET, SPR etc.*
- *AS-MS, Ubiquitination assay*

Degrader profiling

- *Proteomics*
- *Proximity labeling*
- *X-ray crystallography*
- *Degradation pathway*
- *si/shRNA*
- *CRISPR/Cas9 library*

Special Solutions for Bifunctional Degraders

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High-throughput Bifunctional Degrader Synthesis

Degrader chemical toolbox × **Multi-step high-throughput synthesis**

Fast accessible E3 binders & Linkers

> 50 E3 ligase binders *Several thousands linker*

E3 binder × Linker × PoI binder

3-5 steps, 96 compounds/1 batch per 2-3 weeks

Flexible combination** and **Fast access of diverse chemical space

Accelerate hit generation, lead generation** and **optimization

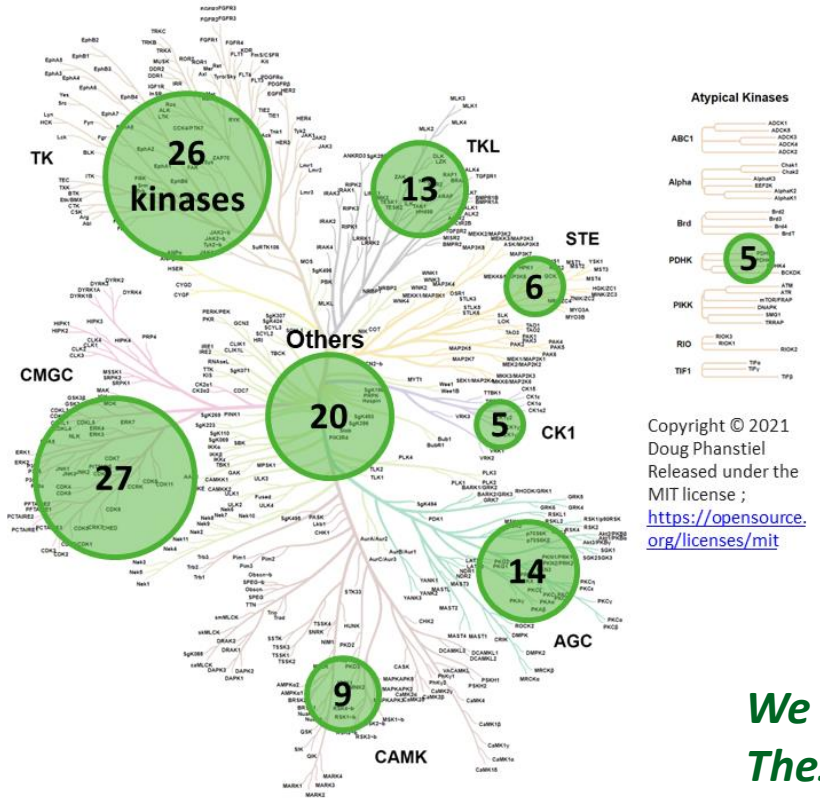
PoI: Protein of interest

Our Original Compound Assets (Protein of Interest Binders)

The number of targets for which binders have been obtained is **>500**.

From ADDP assets (>500 drug target)

Example.



A-SKIP

Axcelead Selective Kinase Inhibitor Profiler

125 kinases

Selective kinase inhibitor, “A-SKIP” criteria
- Selectivity score<=5%
- Highest pIC50>=7.5
- Number of (pIC50>=7.5) <=4

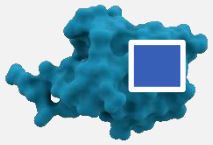
Kinase is one of the important target classes of interest in TPD.

- Many kinases have disease-relevant non-enzymatic functions
- Avoiding or reducing drug resistance
- Axcelead have highly selective kinase inhibitors against 125 kinases

We also have many binders for various target classes.
These could be of great support in the discover of promising bifunctional degraders!

Drug Design of Oral Bifunctional Degrader

Rapid discovery of bifunctional degraders with favorable ADME properties

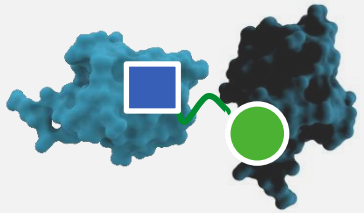


Target binders

Design knowledge

ADME Database
AI/ML systems

E3 binders
& linkers



Bifunctional degrader

Internal study example

Original target X binders

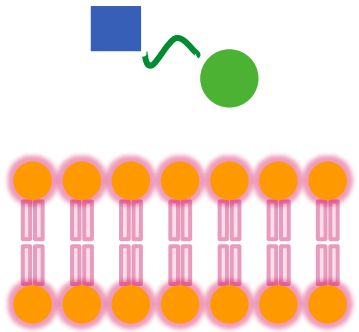
Synthesized ca.60 compounds

>90% *compounds were orally absorbable*

Several compounds ; Oral BA>20%
Acceptable in vitro ADME profile
Confirmed target X degradation

ADME Evaluation of Bifunctional Degraders

In vitro ADMET standard menu



Membrane permeability ×

JP2 solubility ×

Microsomal stability ○

⟺

IVIVC ×

Mouse PK

Oral BA ○

Suitable ADME menu for bifunctional degraders in Axcelead

Solubility

- **JP1**
- **FaSSIF**
- **FeSSIF**

Membrane permeability

- **Customized Caco-2**
- **Customized PAMPA**

Properties

- **EPSA**

Formulation

- **Solubilizing agents**

✓ ***Improving IVIVC***

✓ ***Formulation could dramatically improve oral BA***

IVIVC: In Vitro-In Vivo Correlation

Special Solutions for Molecular Glue Degraders

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- ***MGD-focused*** *compound library*
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Common degrader platform

Screening

- *HiBiT®, Cell imaging assay etc.*
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- *AS-MS, Ubiquitination assay*

Degrader profiling

- *Proteomics*
- *Proximity labeling*
- *X-ray crystallography*
- *Degradation pathway*
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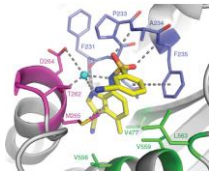
Molecular Glue Degrader Focused Library

6,400 compounds selected from ADDP 1.5M library, which would be suitable for various MGD discovery.

- **Our original binders of several E3 ligases are included.**
- **Mainly, the library were selected by our multiple computational analysis of known MGDs and diverse potential MGD, followed by selection considering structural diversity in each annotation.**

1.5M



- *Original E3 binders*
- *Similarity search*
- *Substructure search*
- *Pharmacophore search*
- *Docking study*

6400 cmpds
Ca.20 annotations

Hit annotation may accelerate to identify the degradation mechanism

MGD/Potential MGD internal database

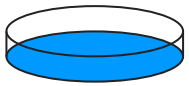
MGD (CRBN, DCAF15, VHL, ATTECs etc,)
E3 ligase binders

Nature Chemical Biology volume 16, pages7–14(2020)

Neo-substrate Identification

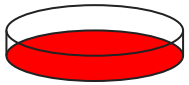
Proteomics

DMSO



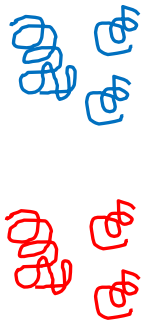
light medium (normal)

Degrader



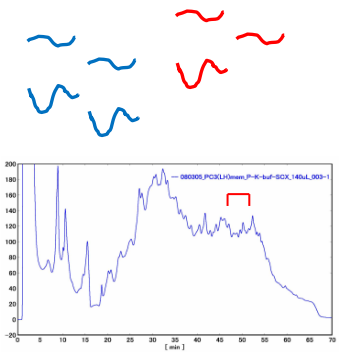
Heavy medium ($^{13}C_6$-Lys, $^{13}C_6$-Arg)

Lysis

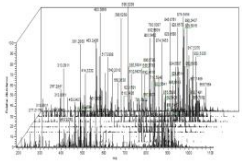


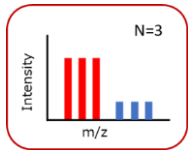
Mix

Trypsin digestion
Fractionation

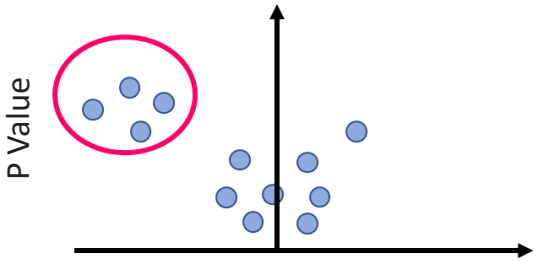


LC/MS analysis



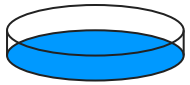


Down-regulated proteins

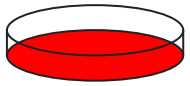


Consensus proteins
⇒ **Neo-substrate candidates**

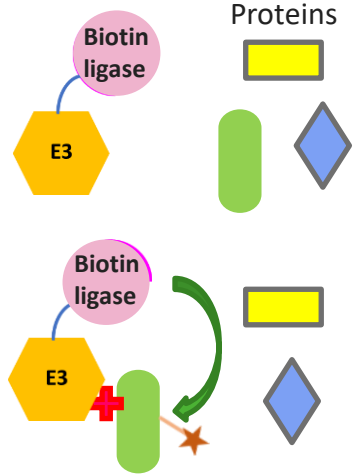
Proximity labeling



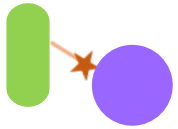
- **Biotin ligase-E3 vector**
- **Biotin**
- **DMSO**



- **Biotin ligase-E3 vector**
- **Biotin**
- **Compound**

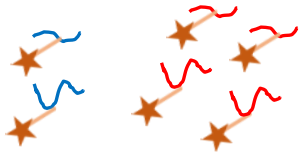


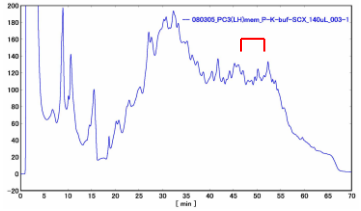
Mix



Streptavidine bead
Pull-down

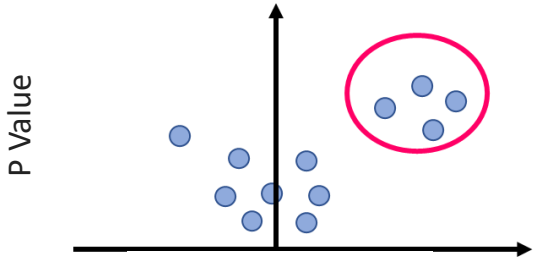
On-beads digestion
Fractionation





LC/MS analysis

Proximity-induced proteins



Evaluation Platform of Targeted Protein Degrader

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Protein Degrader Screening

High-throughput protein quantification assay

HiBiT®

- Large scale high-throughput screening
- HiBiT tag knock-in by CRISPR-cas9

Cell imaging assay (High content screening)

- Highly accurate and sensitive protein quantitation

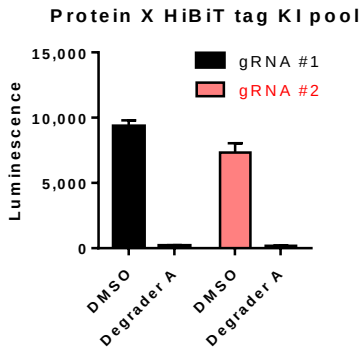
etc.

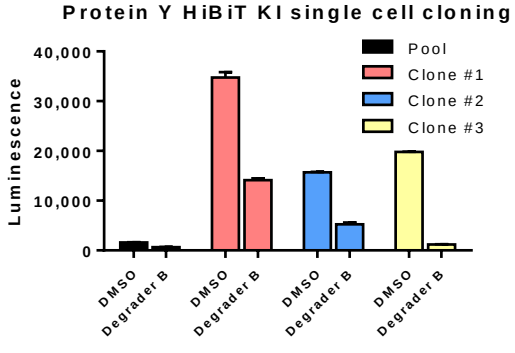
Ternary complex assay

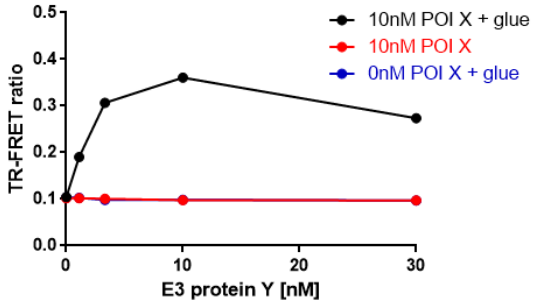
- TR-FRET, AlphaScreen, NanoBRET®, SPR

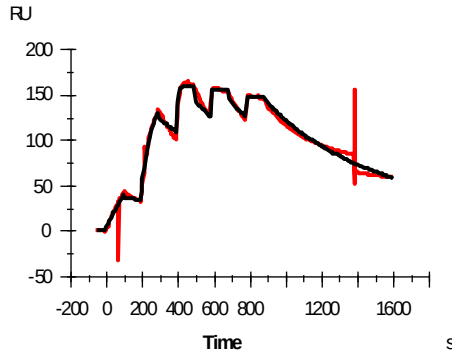
Other TPD-related assays

- E3 ligase binding screening
- AS-MS screening
- Ubiquitination assay

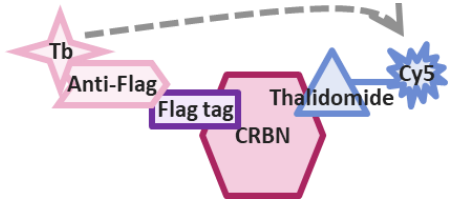


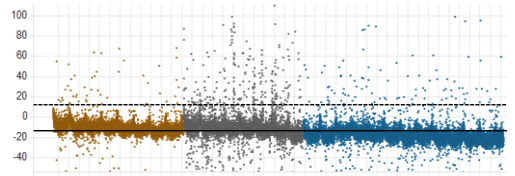






Ternary
Kd; 0.25nM
a = 124





HiBiT & NanoBRET are registered trademarks of Promega corporation.

Protein Degrader Profiling

Items	Material/Platform	Output
E3 ligase- and proteasome-dependency	• ±E3 ligase KD (siRNA) • ±Ubiquitin proteasome system inhibitor(s)	Various
Ubiquitination of PoI	• ±E3 ligase KD (siRNA)	Jess WB
Interaction of E3 and PoI	• Ternary complex (PoI/E3/Cmpd) • Proximity labeling	Jess WB MS
Comprehensive selectivity	• Proteomics • Proximity labeling	MS
Identification of degradation mechanism	• Proximity labeling • CRISPR/cas9 library • Photoaffinity labeling	MS
Structural determination & analysis	• X-ray crystallography	-

PoI: Protein of interest

Our Integrated Services Drive Your Protein Degrader Projects

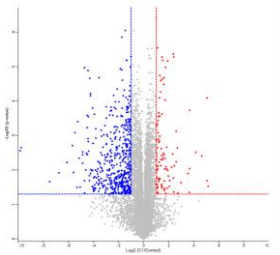
Design
- Expertized medicinal chemists
- AI/ML ADME prediction
- Structure-based drug design
- Original ADME data
- Experts

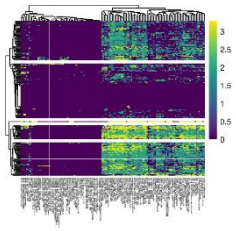
Make
- High-throughput synthesis & purification
- Degrader chemical toolbox
- Enhanced chemical technologies

Analyze
- Structure activity relationship
- Multi-parameter analysis

Test
- Assay & profiling
- High-throughput ADMET
- In vivo evaluation
- PK/PD

Target ID & validation → **Hit generation** → **Lead generation/ Optimization** → **Candidate selection**





Knock-out phenotype
Neo-substrate candidates
E3 ligase selection etc.

Human KI rodent

If you are interested in any of our services, please contact us intl_contact@axcelead.com

Contact Us for Details
E-mail address / Contact form

AXCELEAD
Drug Discovery Partners

Please contact us for any questions!

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intl_contact@axcelead.com

Contact Form
https://axcelead-us.com/contact-us/

Scan to open
our contact form

We value your concerns and questions!